

Name: Coleen Tran

Date: 6/17/14

WEEKLY ONE SENTENCE:

redoing/characterizing RBS + promoter  
in mCherry

DAILY ONE SENTENCE:

all RBS + P<sub>trc</sub>

METHOD(S) OF CHOICE:

PCR  $\Rightarrow$  gel extract  $\Rightarrow$  digest  $\Rightarrow$  ligate

EXPECTED RESULT:

> 1000 bp ✓

☒ check if APE file exists on Drive

which? H<sub>1</sub>-L<sub>1</sub>?

TEMPLATE(S): mCherry + RBS, ~~P<sub>trc</sub>~~

OLIGO(S): P<sub>trc</sub> (81) ✓, 373 ✓

THERMOCYCLER SETTINGS:

94-2'; { 94'-15", 65-15", 68-1.5' } x 31 68°C - 4'; 4°C ✓

CONTROLS:

POSITIVE:

template only? now... no (+ needed)

NEGATIVE:

no polymerase

CONFIRMATION?

gel

GEL LANE ORDER:

GEL PICTURE / OTHER SPACE:

Name: Troy von Beck

Date: 6/17/2014

WEEKLY ONE SENTENCE: Finish initial Wiki HTML code, begin more advanced/better looking formatting.

DAILY ONE SENTENCE: Gel Extract Re-PCR products from Yesterday.  
Create all accessory pages and improve the page links.

METHOD(S) OF CHOICE:

EXPECTED RESULT:

9 Bands of mCherry ~1100 bp  
each with an RBS + Promoter Combo  
^  
unique

☒ check if APE file exists on Drive)

TEMPLATE(S): 9 Bands extracted on Friday

OLIGO(S): Same Oligos used on each respective Band previously

THERMOCYCLER SETTINGS: Same as on Friday

CONTROLS: NTC

POSITIVE: P??/R??/mCherry/VR

NEGATIVE: NTC

CONFIRMATION?

Gel Electrophoresis

GEL LANE ORDER:

GEL PICTURE / OTHER SPACE:

Results: Successfully extracted bands for 2 of the 9  
Promoter/ABS combos PH2/RL1 + PH1L/RM1  
39 57  
ng/ul

Name: Tishi Choudhury

Date: 06/17/19

#### WEEKLY ONE SENTENCER:

Complete at the RBS primer + Promoter primer addition  
Finish crowd-finding things.

#### DAILY ONE SENTENCER:

Digest PCR amplification if gel shows up nicely. Also add in the promoter to RBS primer & PCR

#### METHOD(S) OF CHOICE:

- PCR, gel extraction & purification (Prom L1)
- Digestion & ligation & amplified the promoters & RBS; + Transfection

#### EXPECTED RESULT:

Good concentrations on nanodrop for the Promoter addition

Also bands  $\sim 1100$  bp

☒ check if APE file exists on Drive

#### TEMPLATE(S):

RBS: H1, H2, M1, M2 & L1 inserted in front of mCherry

#### OLIGO(S):

273, PL1

#### THERMOCYCLER SETTINGS:

65°

94-2', 94-15", 65-15", 68°C-1.5'  $\times 31$  - 68°C-4', 4°C forever

#### CONTROLS:

POSITIVE: With polymerase

NEGATIVE: No polymerase

#### CONFIRMATION?

Running a gel & finding  $> 1000$  bp bands

#### GEL LANE ORDER:

For mCherry + RBS + Prom: L1, B1, B2, B3, B4, B5, B6, E1, no data

#### GEL PICTURE / OTHER SPACE:

ADD PCR for 35 combinations.

Primer	RH1	RH2	RM1	RM2	PL1
PH1	A1	B1	C1	D1	E1
PH2	A2	B2	C2	D2	E2
PM1	A3	B3	C3	D3	E3
PM2	A4	B4	C4	D4	E4
PL1	A5	B5	C5	D5	E5
PL2	A6	B6	C6	D6	E6

for negatives: A1 - B6 with no polymerase and primer PH1.

\* Adding is ligation.

\* Did ligation mCherry with RBS & the mCherry backbone  
transformed using Durolight & our comp.

100 bp mCherry RBS

The end.

Name: Stefan Tassoulas

Date: 06/17/14

WEEKLY ONE SENTENCE:

Characterize rbs & promoter pairs in E. coli:

Work on finding (Morgan) →

DAILY ONE SENTENCE:

Insert Promoter in rbs machinery insert

METHOD(S) OF CHOICE:

PCR  
Gel extraction

EXPECTED RESULT:

~979 bp promoter-rbs-mCherry✓

(☐ check if APE file exists on Drive)

TEMPLATE(S): RBS(H1-L2) mCherry

OLIGO(S): 086 (P22): 0863 (VR)✓

THERMOCYCLER SETTINGS: 94-2' 94' -15' 65-15' 68-1.5' 68-4' 40°C

CONTROLS:

POSITIVE:

w/ polymerase ✓

NEGATIVE:

w/o polymerase ✓

CONFIRMATION?

Strong band at ~979 bp Gel

GEL LANE ORDER:

16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

Gel extract (this will be on multiple gels → ladders w/ picking file names is essential)

GEL PICTURE / OTHER SPACE:

011 49 21 03 2900